Amendments to the Claims

Please cancel claims 1-26, 28-59, and 61-78 without prejudice and add claims 79-87. This listing of claims will replace all prior versions and listings of claims in this application.

1.–26 . (Canceled)

27. (Currently amended) A method for producing a ~~modified~~ chimeric mouse ~~non-human animal~~, said ~~animal~~ chimeric mouse having a fragment of an unrearranged human immunoglobulin heavy chain locus sufficient to encode a human immunoglobulin heavy chain and a fragment of an unrearranged human immunoglobulin light chain locus sufficient to encode a human immunoglobulin heavy chain, wherein the fragment of an unrearranged heavy chain locus and the fragment of an unrearranged light chain locus are stably integrated into the same chromosome in at least some of its cells, the method comprising the steps of:

(a) combining under fusing conditions a mouse ES cell and a yeast spheroplast, wherein said spheroplast contains two or more yeast artificial chromosomes (YAC), wherein at least one YAC comprises the fragment of an unrearranged human immunoglobulin heavy chain locus and at least one YAC comprises the fragment of an unrearranged human immunoglobulin light chain locus, and wherein each YAC includes a gene encoding a selectable marker, whereby the fragments become stably integrated into the genome of the ES cell;

(b) selecting for an ES cell carrying the human immunoglobulin loci by means of one or more of the markers; and

(c) producing the chimeric mouse from said ES cell carrying the human immunoglobulin loci.

~~a xenogeneic DNA segment of at least 100 kb stably integrated into the genome of said animal, said method comprising:~~

~~combining under fusing conditions yeast spheroplasts, said spheroplasts comprising a YAC having said xenogeneic DNA segment and a marker for selection, with embryonic stem cells of said animal, whereby said xenogeneic DNA segment becomes integrated into the genome of said embryonic stem cells;~~

~~selecting for embryonic stem cells carrying said xenogeneic DNA segment by means of the marker;~~

~~transferring said embryonic cells into a host blastocyst and implanting said blastocyst in a pseudopregnant animal recipient, and allowing said blastocyst to develope to term to produce a chimeric animal carrying said xenogeneic DNA segment; and~~

~~mating said chimeric animal with an animal of the same species to produce said modified animal carrying said xenogeneic DNA segment.~~

28.-59. (Canceled)

60. (Currently amended) A method of producing a mouse embryonic stem (ES) cell having a fragment of an unrearranged human immunoglobulin heavy chain locus sufficient to encode a human immunoglobulin heavy chain and a fragment of an unrearranged human immunoglobulin light chain locus sufficient to encode a human immunoglobulin light chain, wherein the fragment of an unrearranged heavy chain locus and the fragment of an unrearranged light chain locus are stably integrated into the same chromosome of the mouse ES cell, the method comprising the steps of:

(a) combining under fusing conditions a mouse ES cell and a yeast spheroplast, wherein said spheroplast contains two or more YACs, wherein at least one YAC comprises the fragment of an unrearranged human immunoglobulin heavy chain locus and at least one YAC comprises the fragment of an unrearranged human immunoglobulin light chain locus, and wherein each YAC includes a gene encoding a selectable marker, whereby the fragments become stably integrated into the genome of the ES cell; and

(b) selecting for an ES cell carrying the human immunoglobulin loci by means of one or more of the markers ~~for modifying a genome of a recipient murine embryonic stem cell by homologous recombination with a large xenogeneic DNA genomic fragment previously manipulated in a yeast artificial chromosome (YAC), the improvement which comprises:~~

~~introducing at least one YAC into saidmurine embryonic stem cell by spheroplast fusion, and selecting recipient cells comprising said genomic fraqment, wherein said YAC comprises a mammalian selectable or screenable gene, wherein said YAC is faithfully transmitted through the host germline, and said xenogeneic DNA fragment is transmitted in substantially intact form~~.

61-78. (Canceled)

79. (New) A method of producing a transgenic mouse and its progeny having a fragment of an unrearranged human immunoglobulin heavy chain locus sufficient to encode a human immunoglobulin heavy chain and a fragment of an unrearranged human immunoglobulin light chain locus sufficient to encode a human immunoglobulin light chain, wherein the fragment of an unrearranged heavy chain and the fragment of an unrearranged light chain are stably integrated into the same chromosome in its somatic and germ cells, the method comprising the steps of:

(a) combining under fusing conditions a mouse ES cell and a yeast spheroplast, wherein said spheroplast contains two or more YACs, wherein at least one YAC comprises the fragment of an unrearranged human immunoglobulin heavy chain locus and at least one YAC comprises the fragment of an unrearranged human immunoglobulin light chain locus, and wherein each YAC includes a gene encoding a selectable marker, whereby the fragments become stably integrated into the genome of the ES cell;

(b) selecting for an ES cell carrying the human immunoglobulin loci by means of one or more of the markers;

(c) producing a chimeric mouse from said ES cell carrying the human immunoglobulin loci; and

(d) breeding said chimeric mouse and its progeny to produce the transgenic mouse.

80. (New) The method according to any one of claims 27, 60, or 79, wherein the fragment of an unrearranged human immunoglobulin heavy chain locus is a DNA sequence substantially identical to the germline DNA sequence of human chromosome 14 from the D segment genes of the human immunoglobulin heavy chain locus, continuing through the J segment genes and the constant region genes through Cμ of that locus, wherein said DNA sequence does not include a gamma constant region, and wherein said DNA sequence is operably linked to at least one human V segment gene.

81. (New) The method according to any one of claims 27, 60, or 79, wherein the fragment of an unrearranged human immunoglobulin heavy chain locus is a

DNA fragment, said fragment consisting essentially of a SpeI-SpeI fragment commencing at the VH6 gene and continuing through the human D segment genes, human J segment genes and human constant region genes and into the Cδ gene of that locus, wherein said SpeI-SpeI fragment does not include a gamma constant region.

82. (New) The method according to any one of claims 27, 60, or 79, wherein the fragment of an unrearranged human immunoglobulin heavy chain locus is a DNA fragment, said fragment consisting essentially of a SpeI-SpeI fragment commencing at the VH6 gene and continuing through the human D segment genes, human J segment genes and through the human Cμ constant region gene said SpeI-SpeI fragment terminating within the Cδ gene of that locus.

83. (New) The method according to any one of claims 27, 60, or 79, wherein the fragment of an unrearranged human immunoglobulin heavy chain locus is a DNA sequence consisting essentially of 85-100 kb of human chromosome 14, extending from within the V segment genes of the human immunoglobulin gene locus at a position 3′ of a SpeI restriction site through the human Cμ constant region gene.

84. (New) The method according to any one of claims 27, 60, or 79, wherein the fragment of an unrearranged human immunoglobulin heavy chain locus is a DNA sequence consisting essentially of between 85-100 kb extending from the human D segment genes through the human Cμ constant region, wherein the DNA sequence is operably linked to at least one human V segment gene.

85. (New) The method according to any one of claims 27, 60, or 79, wherein the genome of the ES cell comprises at least one inactivated endogenous immunoglobulin heavy chain locus in which all of the J segment genes are deleted to prevent rearrangement of the locus and to prevent formation of a transcript of a rearranged endogenous immunoglobulin heavy chain locus.

86. (New) The method according to any one of claims 27, 60, or 79, wherein the genome of the ES cell comprises at least one inactivated endogenous immunoglobulin light chain locus in which the Cκ gene is deleted to prevent rearrangement

of the locus and to prevent formation of a transcript of a rearranged endogenous immunoglobulin light chain locus.

87. (New) The method according to any one of claims 27, 60, or 79, wherein the genome of the ES cell comprises two inactivated endogenous immunoglobulin heavy chain loci in which all of the J segment genes are deleted in both loci, or two inactivated endogenous immunoglobulin light chain loci in which the Cκ gene is deleted in both loci, or two inactivated endogenous immunoglobulin heavy chain loci in which all of the J segment genes are deleted in both loci and two inactivated endogenous immunoglobulin light chain loci in which the Cκ gene is deleted in both loci.